Taxonomic Classification and Phylogenetic analysis of Ants (*Hymenoptera: Formicidae*) and deciphering economic importance of Ants from Painganga Wildlife Sanctuary

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By

Mr. Sumeet Govindrao Dhondge

Mobile No. +91-9503386014 | Email: dhondgesumeet123@gmail.com

Under the Supervision of

Dr. Arvind B. Chavhan

Assistant Professor and Head,Department of Zoology

Digambarrao Bindu College Bhokar, Dist Nanded

Mobile No. +91-9420775527 | Email: drarvindchavhan@gmail.com

Research Center

Department of Zoology,

Shri Shivaji College, Parbhani, MS, India

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1. INTRODUCTION

Ants, being social insects, play a crucial role in ecosystems, including wildlife sanctuaries like the Painganga Wildlife Sanctuary. The economic importance of ants in the Painganga Wildlife Sanctuary is intertwined with their ecological roles, affecting soil health, pest control, plant growth, and contributing to the overall balance of the ecosystem. Understanding and conserving ant populations can have both direct and indirect economic implications for the local environment and communities. The proposed study outlooks approach to provide an overview of ants and their ecological significance. Study also highlights the vast diversity of ants, emphasizing their role in ecosystems. Study also provides a scientific basis for the need for a comprehensive taxonomic classification and Phylogenetic analysis to better understand ant diversity. The economic importance of ants in such habitats can be diverse and multifaceted:

Soil Aeration and Nutrient Cycling:

- Ants are known to be ecosystem engineers, contributing significantly to soil aeration through their burrowing activities.
- They enhance nutrient cycling by breaking down organic matter and recycling nutrients back into the soil, positively influencing plant growth.

Seed Dispersal:

- Certain ant species are involved in seed dispersal, aiding in the regeneration of plant populations.
- This can be particularly important for maintaining biodiversity and supporting the growth of vegetation, which can have indirect economic benefits for the local ecosystem.

Pest Control:

- Ants are natural predators, and many species contribute to pest control by preying on insect larvae and other pests.

- This can reduce the need for chemical pesticides, potentially leading to economic savings in agriculture and forestry practices.

Pollination:

- Some ant species play a role in pollination, especially in association with certain plant species.
- This contributes to the reproduction of plants, including those with economic value, such as certain fruits and crops.

Biodiversity Indicator:

- The presence and diversity of ant species can serve as indicators of overall ecosystem health.
- Monitoring ant populations can help assess the impact of environmental changes, aiding in the conservation and management of biodiversity.

Ecotourism:

- Ants, with their diverse behaviors and ecological roles, can be of interest to ecotourists, contributing to the local economy through tourism activities.
- Educational programs and guided tours focused on ants and their habitats can attract visitors and generate revenue.

Scientific Research and Education:

- Studying ant populations in the Painganga Wildlife Sanctuary can contribute to scientific knowledge about biodiversity, ecology, and behavior.
- Educational programs based on ant ecology can be developed, promoting environmental awareness and contributing to a better understanding of ecosystems.

Traditional Medicine and Ethnobiology:

- In some cultures, ants have been used in traditional medicine for various purposes.
- Studying the ant species in the sanctuary may uncover potential medicinal properties or traditional uses that could have economic implications

Erosion Control:

- Ants activities, such as constructing nests and burrows, can contribute to soil stabilization and erosion control.

- This can have economic benefits for agriculture and infrastructure by preventing soil erosion and maintaining soil structure.

2. REVIEW OF LITERATURE AND DEVELOPMENT IN THE SUBJECT

International status

Insects like bees, wasps, and sawflies are also part of the Order Hymenoptera, which includes ants. All known ant species belong to one of 21 subfamilies within the Formicidae family. In addition, more than 12,000 species of ants have been identified, and there are likely many more that are still waiting to be classified. Entomologists from all across the globe have already identified more than 300 different genera.

Identification of an organism up to the species level is a difficult task and it requires specialized knowledge (Monaghan et al. 2005). Using only a single taxonomy approach i.e. either morphological or molecular approaches has faced criticism in the identification of species (Knowlton 1993; Jarman & Elliott 2000; Rubinhoof 2006). Therefore, the incorporation of both morphological and molecular approaches leads to taxonomic constancy (Padial et al. 2010). As molecular approach DNA barcoding is a very effective tool for species identification of insects (Kumar et al. 2012), it contributes a new direction for species-level identification among invertebrates and vertebrates (Hebert et al. 2004; Hajibabaei et al. 2006).

A tradition of studies in comparative ant morphology during the second half of the 20th century produced an extensive set of published work. Some of the systematic surveys covering particular structures or life stages across all ant taxa include: proventriculus (e.g., Eisner and Wilson, 1952; Eisner, 1957); mouthparts (e.g., Gotwald, 1969; Gotwald, 1970; Gotwald, 1973); sting apparatus (e.g., Kugler, 1991, 1992); strigil (e.g., Francoeur and Loiselle, 1988); and larvae (e.g., Wheeler and Wheeler, 1960). Although the wealth of illustrations accompanying these studies remain an invaluable source of morphological information, incorporating these legacy data, produced independently by many different authors over a period of half a century, into a phylogenetic analysis can become an insurmountable problem, not least because the descriptive part of the work requires thorough reinterpretation. Of all the types of studies mentioned above, phylogenetic analyses provide the best way to summarize, document, and disseminate comparative data through the translation of anatomy into discrete character states (Ramírez et al., 2007). Ideally,

morphological data should be no different from molecular-sequence data in terms of how easily it can be retrieved from previously published work and combined with newly generated data to perform phylogenetic analyses covering a wider range of taxa or previously unexplored groups. In practice, it is seldom the case that morphological data can be seamlessly integrated from one study to the next without the need to revisit the original specimens. Additionally, the complexity of morphology in comparison with molecular sequences means that the former often requires a reinterpretation of the anatomical observations in the light of a new or a different combination of taxa (Rieppel and Kearney, 2002; Jenner, 2004a).

2.2 National status

Himender Bharti (2015) India is home to an extraordinary array of plant and animal life, ranking among the world's 17 "megadiverse" nations and including four "biodiversity hotspots" in its territory. There are 828 species and subspecies recorded, from a total of 100 different Indian genera. Possible sources of error, incorrect identifications, and questionable distributional records in the literature are also pointed out. The current comprehensive listing of Indian ants will provide researchers a bird's-eye perspective of diversity and distribution, as well as highlight large, under-sampled regions that might benefit from further sampling and taxonomic attention.

Bhoje et al., (2014) Study is based on species biodiversity conducted at Amba reserve forest collected 65 ant species, in 35 genera. Mahalakshmi and Channaveerappa (2016) their study show that a total of 978 sampled specimens were captured in the study area. The 20 ant species are belonging to 12 genera and four subfamilies.

Chavhan and Pawar, (2011) reported the first comprehensive study on the ants from Amravati city and the allied region, who reported 34 species in 20 genera belonging to five subfamilies. Similarly Dhote (2014) reported a total of 16 species in five subfamilies (Ponerinae, Pseudomyrmecinae, Myrmicinae, Dolichoderinae and Formicinae) and 14 genera from the Amravati region.

2.3 Regional status

Due to a lack of prior taxonomic study in the chosen study area, the results of this study will be groundbreaking for both the region and the researcher. Research on Ants as a species

that is highly relevant, critically required, and consistent with modern societal norms. Proposed research activity will directly address the taxonomic barrier that inhibits the evaluation of pollinator status and pollination services at the present time.

3. ROTATIONAL AND SIGNIFICANCE OF THE STUDY

Since there have been no previous Morphological and Molecular Characterization based taxonomic studies on of this superfamily in Painganga Wild life Sanctuary, the proposed study will be groundbreaking for the area and the researcher alike and will aid in the accumulation of necessary data. Studying Ants at the species level. Species, genera, and families will be separated using the taxonomic keys that will be developed. Some ecological details are recorded, such where and when specimens were collected and which plants they feed on. In order to better understand bee biodiversity, ecology, and conservation, this project intends to catalogue the bee fauna of Painganga Wild life Sanctuary.

4. OBJECTIVES OF RESEARCH / PROPOSED HYPOTHESIS

Due to the lack of Taxonomic Classification and Phylogenetic Analysis of Ants (Hymenoptera: Formicidae) from Painganga Wild Life Sanctuary, so the aims of present works are:

- To find out ant composition and biodiversity from Painganga wildlife Sanctuary.
- To study the Morphology of ants (Hymenoptera: Formicidae) by morphometry.
- To study the Molecular characteristics of ants (Hymenoptera: Formicidae) by DNA sequence.
- To determine phylogeny of ants (Hymenoptera; Formicidae) species in Painganga wildlife sanctuary
- To develop a comprehensive taxonomic framework for ant species, considering both morphological and molecular characteristics.
- To conduct a phylogenetic analysis to elucidate the evolutionary relationships among major ant taxa.

5. METHODOLOGY TO BE ADOPTED

Accessibility and fit within an ecoregion will be taken into account while selecting survey locations. In this analysis, representative species will serve as the final taxonomic level. Several theoretical and practical arguments will be made in favour of using exemplar species as terminals in phylogenetic analyses rather than supraspecific ground plans.

5.1 Study Area /Site Selection

The Painganga Wildlife Sanctuary is much more than just an animal sanctuary; it offers a wide variety of activities, lush scenery, and intriguing geographic features. It would be difficult to resist returning to this 325 square kilometer nature refuge time and time again given the abundance and diversity of its rich and varied flora and animals. The wildlife sanctuary's name originates from the fact that the Painganga or Penganaga Rivers surround it on three sides. A broad valley that separates the districts of Nanded and Yavatmal lies at the center of the sanctuary. The vibrant variety of flora and wildlife, in addition to the landscapes, adds even more appeal to the Painganga Wildlife Sanctuary visit for tourists.

Sampling Site:

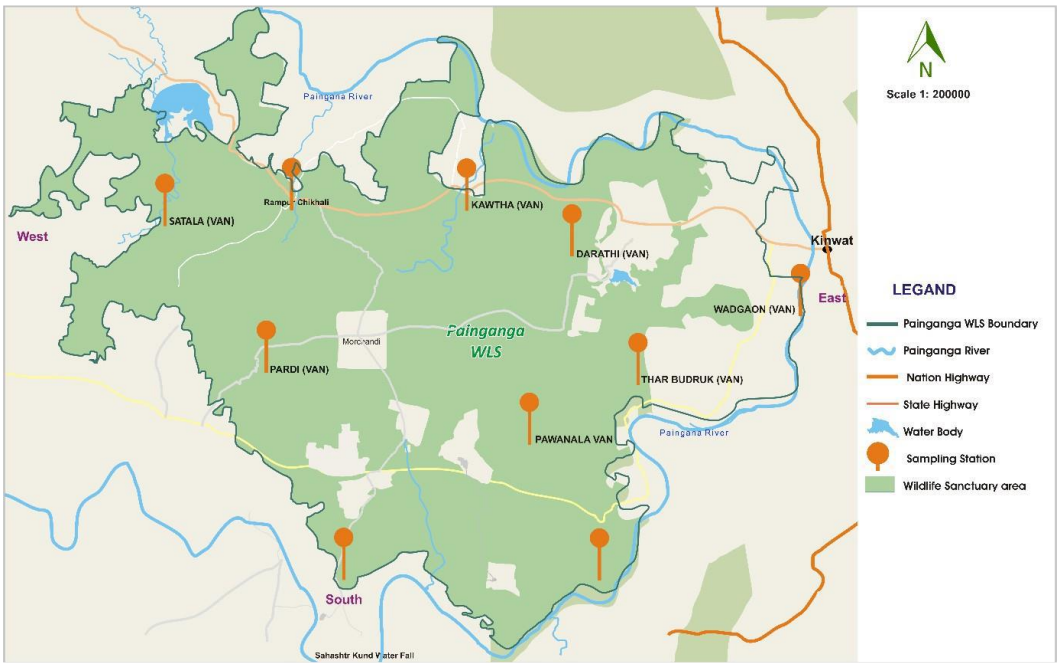

Figure 1: Showing Purpose study site within Painganga wild life sanctuary in Yavatmal and Nanded forest Circle Maharashtra India

5.2 Collection of Ants and Preservation

5.2.1. Pitfall traps

Survey sites will be systematically chosen based on accessibility, location within an ecoregion. At each selected site, a modification of the ants of leaf litter (ALL) protocol will be follow (Bharti et al. 2016). Pitfall traps will be used to sample surface-dwelling ants. Pitfall traps will be placed along each of the 100 m transects at 10 m intervals.

5.2.2. Litter samples:

Two transects (each 300 m in length) will be placed 1 m away from each pitfall trap transect.

5.2.3. Hand collection

Hand collection will be consisted of thoroughly searching vegetation above litter level immediately before litter will be collected and on the bare ground immediately after litter.

5.2.4. Preservation of Collected sample

The contents from all samples will be taken to the laboratory, processes, sorted, and counted and will be preserved in 95% alcohol as per standard protocols (Smith *et al.* 2007, Bharti et al., 2016)

5.3 Morphological Characterization

The functional group of each ant species will be documented (Andersen 1997a). Identification will be performed using stereo zoom microscope based on literature (Mathew and Tiwari, 2000; Tiwari *et al*. 1998; Bolton, 1994; Holldobler and Wilson, 1990; Bingham, 1903, Pacheco 2007). The morphological measurement and indices of ant workers will done using standard measurements given by Leong et al. (2017) with modifications.

5.4 Molecular Characterization

The molecular taxonomy was done into the following steps

5.4.1 DNA Isolation

DNA from ants species will be extracted using a DNA Isolation Kit (Invitrogen) using a modified tissue protocol from their manual (Smith *et al.* 2007). Standard primers will be used for sequence the mitochondrial cytochrome oxidase-I (COI). Extracted DNA will be store at 4.0^{O}C until PCR amplification is done.

5.4.2 PCR Amplification and DNA Sequencing

PCR Amplification and DNA sequencing will be done by availing hiring services. The PCR program will be optimized as per standard protocols of Smith et al., (2007).

5.4.3 Phylogenetic analysis

COI region sequences obtained from investigated ant samples were assembled using ChromasProV3.1 sequence assembly software. The assembled sequences were used for BLAST analysis using multiple sequence analysis tools available at the NCBI database.

5.5 Data Analyses:

- Ant species listed and the complete count of the number of species presented in each habitat will be done for species composition and species structure indices (Krebs, 1999).
- Morphological Varion among different ants Taxon's will be compared
- Molecular phylogenetic analysis will be done for multilocus dataset based on Bayesian and maximum likelihood (ML) methods.
- All statistical analyses were performed using SigmaStat 2.0 by SPSS Inc. 1997).

6. FACILITIES REQUIRED AND AVAILABLILITY

To carried out the proposed research work department of Zoology, Digambarrao Bindu Arts Commerce and Science Bhokar, Has Following facilities,

1. Expertise in Ant Taxonomy
2. Zoological Laboratory has Stemi DV4 Stereozoom Microscope (Zeiss) used for the Morphological characterisation.
3. Sampling Devises and Ambient Laboratory is available to preserved collected sample.

Apart from the morphological characterization department has entered in the in the MOU with MetaGenBio.Lab for the Molecular Characterization of the collected Specimens.

Facilities available with MetagenBio.Pvt.Ltd

1. DNA Isolation Kit.
2. PCR Thermal Cyclers Applied Biosystems
3. DNA sequencing Applied Biosystems 3130 Genetic Analyzer e an automated DNA sequencing machine.

7. YEAR WISE PLAN OF WORK

Year-wise plan of work

1st year: -

7.1.1 Review and Literature

7.1.2 Gap Analysis of the Region and Site selection for sample

7.1.3 Adoption of sampling methods as per site requirements species diversity

7.1.4 Collection of ants using appropriate sampling methods following standards protocol from Painganga wild life sanctuary.

7.1.5 Sorting of Ants according to the morphological Characters/Phenotype

7.1.6 Identification of ant species as per the generated key based on morphological Characters/Phenotype

2nd year: -

7.2.1 Collection of ants using appropriate sampling methods following standards protocol from Painganga wild life sanctuary.

7.2.2 Sorting of Ants according to the morphological Characters/Phenotype

7.2.3 Study a different paradigm that uses digital images for character assessment and documentation.

7.2.4 Morphological study of ants using morphometry.

7.2.5 Molecular Characterization of Identified Ants Species.

7.2.6 Publication of Research work

3rd year: -

7.3.1 To study the context of phylogeny makes it easy to analyse and compare the morphological observation.

7.3.2 To study the value of ants economically in the Painganga Wildlife Sanctuary

7.3.3 Completion of research work

7.3.4 Thesis writing

7.3.5 Publication of research work

7.3.6 Final Submission of thesis

8. EXPECTED OUTCOMES

This study will demonstrate both the morphological and molecular approaches are helpful for the identification of ant species. The integration between morphological and DNA data into simultaneous phylogenetic analysis, our present method of ant categorization is more diagnostic than phylogenetic. Since phylogeny is the common pattern underlying organismal variety, it is the greatest approach to summarise biological data, and progress towards will achieve a phylogenetic classification will only improve the accuracy of diagnosis in our classification system.

Present study will also intend in achieving objectives of ZSI i.e.

- Will help in preparation of Red Data Book,
- Bioecologically studies on selected important communities/species.
- Preparation of Morphological and Molecular databases for the recorded species from the Painganga wild life Sanctuary.
- Publication of results generated out of proposed research will also be one the outcome
- Digitization of the recorded species will be done after the completion proposed research works.

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Research Scholar

Dhondge Sumeet Govindrao

Research Supervisor

Dr. Arvind B Chavhan